VCAN – A novel prognostic marker for gastric cancer

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Abstract

Background Versican (VCAN) is a large aggregating extracellular matrix proteoglycan implicated in the pathogenesis of most human cancers but its role in gastric cancer is not yet elucidated. We designed the present study to investigate the expression, prognostic value, relationship between genetic alterations and patient's outcome, disease associations, as well as genetic and protein interactions of VCAN in gastric cancer. Methods Expression of VCAN in tumor and normal tissues was studied with ONCOMINE, UALCAN, and GEPIA databases and the human protein atlas. UALCAN, GEPIA, OncoLnc and Kaplan-Meier plotter were used to assess the prognostic values of VCAN in gastric cancer. Subgroup analysis of the clinical significance of VCAN in gastric cancer was done using Kaplan-Meier plotter. Genetic alterations of VCAN and their associations with patient's outcome were studied with cBioPortal. Open targets platform was used to study diseases associated with VCAN, GeneMANIA was used to obtain the neighbor genes interaction network of VCAN, and STRING was used to examine VCAN interaction with other proteins. Results VCAN was upregulated and associated with clinical cancer stages. High VCAN expression predicted unfavorable outcome for all patients; subgroup analysis showed worse outcomes for patients treated with surgery or other adjuvants (other than 5-FU based adjuvant) but better outcome for patients treated with 5-FU based adjuvant, associating VCAN expression with chemosensitivity to 5-FU based adjuvants, but chemoresistance to other adjuvants. Genetic alteration of VCAN correlated with a favorable outcome in terms of disease-free survival for patients. Also, open targets platform showed that VCAN is associated with gastric cancer and is a potential therapeutic target for the disease. Finally, interaction of VCAN with neighbor genes and proteins revealed that VCAN interacts with genes and proteins that drive tumorigenesis and cancer progression. Conclusion The results of this study show that VCAN is an oncogene in gastric cancer whose expression can be applied as a biomarker for prognostic prediction, selection of appropriate chemotherapy drugs/monitoring of treatment response, and is a potential therapeutic target for gastric cancer.

Background

The discovery of genes that contribute to cancer has been an extremely important development for cancer research. This is because carcinogenesis is mostly genetic and majority of cancers are observed to have some type of genetic alteration(1). Overwhelming evidence has it that the provision of lasting panacea for human cancers lies in the identification of tumor associated genes which can be markers of patient's outcome and targets for therapy(2–5). So far, aberrant gene expressions have been implicated to cause malignant signatures in gastric cancer and abnormally expressed genes are thought to be potential tumor markers and therapeutic targets(6–8). Identification of genes that fuel tumorigenic processes is pertinent to the development of personalized therapies and disease management plans.

Versican (VCAN), a member of the large chondroitin sulfate proteoglycan family that interacts with hyaluronan is a large aggregating extracellular matrix proteoglycan, about 900 kDa, with 15-20 chondroitin sulfate side chains(9,10). It has several active domains that influences its interactions with certain biological and pathological processes, and functions in extracellular matrix assembly and

remodeling, anti-adhesion, as well as tumor cell proliferation, and migration(11). It is suggested to be involved in the development and progression of several malignancies due to its frequent expression in human cancers(12), and several studies have also validated its role in carcinogenesis - it is implicated in the pathogenesis of colorectal cancer, breast cancer, benign prostatic hyperplasia, skin cancer, oral squamous cell carcinoma, early-stage prostate cancer, and in leiomyomas, amongst others and associated with survival outcomes(11,13–18). However, its role in gastric cancer is not yet explained. In this study, we investigated the expression and clinical significance of VCAN to explicate its role and potential as a biomarker for gastric cancer.

Materials And Methods

Ethics statement

Our study was approved by the Ethics Committee of the Second Hospital of Shandong University. Since all data were obtained from publicly accessible online databases, all written informed consent would have already been obtained.

ONCOMINE, UALCAN and GEPIA analyses

The mRNA levels of VCAN in 20 different cancer types were determined through analysis in ONCOMINE database (www.oncomine.org)(19). Expression of VCAN in primary tumor tissues and normal tissues were validated in UALCAN (http://ualcan.path.uab.edu)(20) and gene expression profiling interactive analysis (GEPIA) (http://gepia.cancer-pku.cn)(21) databases. Difference of transcriptional expressions was compared by students' t test and $P < 0.05$ was considered as statistically significant.

The Human Protein Atlas

The human protein atlas is a website that contains immunohistochemistry-based expression data for 20 most common kinds of cancers(22). Direct comparison of protein expression of VCAN between normal human stomach tissue and gastric cancer tissue was examined by immunohistochemistry image on the human protein atlas (https://www.proteinatlas.org).

Survival analysis

Datasets from four independent cohorts were utilized for analysis of VCAN expression as a prognostic factor for gastric cancer, namely: UALCAN, GEPIA, OncoLnc (www.oncolnc.org/)(23), and Kaplan-Meier plotter (http://kmplot.com/analysis/)(24–26) (Affymetrix ID: 204619_s_at). Subgroup analysis was performed using Kaplan-Meier plotter, and the survival analysis results of 876 gastric cancer tissue samples from The Cancer Genome Atlas (TCGA) are available at the Kaplan Meier plotter database. $P < 0.05$ was considered as statistically significant.

CBioPortal analysis

Gene alteration frequency of VCAN in stomach adenocarcinoma (STAD) was performed using the CBioPortal for Cancer Genomics (http://www.cbioportal.org)(27) database. Genetic mutations in VCAN and its association with disease-free survival (DFS) was displayed as Kaplan-Meier plots and log-rank test.

Open Targets Platform

Diseases associated with VCAN based on pathways and systems biology were examined using the Open Targets Platform (www.targetvalidation.org/) which is a comprehensive and robust data integration platform for access to and visualization of potential drug targets associated with diseases(28).

GeneMANIA and STRING analyses

GeneMANIA (http://www.genemania.org)(29) was used to conduct correlation analysis of VCAN at the gene level. STRING (https://string-db.org/)(30) database was used to conduct correlation analysis of VCAN at the protein level.

Results

VCAN is upregulated in gastric cancer and correlates with cancer stage:

To examine the role of VCAN in gastric cancer, we first studied its expression in tumor tissues and normal tissues using three independent cohorts – ONCOMINE, UALCAN, and GEPIA datasets. VCAN was upregulated in all three datasets (Figure 1A – 1C). Pan cancer analysis in ONCOMINE showed that VCAN is upregulated in most human cancers and shows that currently, seven independent datasets report VCAN upregulation in gastric cancer (Figure 1A). Expression of VCAN also correlated with tumor stage, expression increased with increase in stage and the highest expression was found in the most advanced cancer stage (stage 4) (Figure 1B – 1C). We also examined the protein expression of VCAN in normal stomach and gastric cancer using the human protein atlas. VCAN was more highly expressed in cancer tissues than normal tissues as indicted by high antibody staining in cancer and medium antibody staining in normal tissues (Figure 1D). Together, these results show that VCAN is upregulated in gastric cancer and correlates with cancer stage.

High VCAN expression predicts worse outcomes for patients; and VCAN expression is associated with response to chemotherapy:

Using four independent datasets, we studied the prognostic value of VCAN in gastric cancer. High VCAN expression correlated with poor prognosis for patients in all four datasets (all $P < 0.05$) (Figure 2A – 2D). Subgroup analysis in Kaplan-Meier plotter database showed that shorter OS and PPS were found in all patients (HR=1.32, P=0.0033, and HR=1.37, P=0.0086 respectively), intestinal type patients (HR=2, P=2.4e-05, and HR=1.81, P=0.0044 respectively), diffuse type patients (HR=1.77, P=0.00097, and HR=1.72, P=0.0045respectively), patients treated with surgery only (HR=1.9, P=1.1e-05, and HR=1.77, P=0.00026 respectively), or other adjuvant (other 5-FU based adjuvant) only (HR=3.04, P=0.0091 and

HR=2.72, P=0.021 respectively). However, longer OS and PPS times were observed in patients treated with 5-FU based adjuvant only (HR=0.63, P=0.023, and HR=0.56, P=0.0059 respectively) (Figure 3A-3F). Together these results show the prognostic value of VCAN in gastric cancer, and its association with response to chemotherapy.

Genetic alteration in VCAN is a marker of favorable disease-free survival (DFS) for patients:

Previous studies have linked alterations in certain genes with carcinogenesis(31). We investigated possible associations between genetic alteration in VCAN with disease-free survival (DFS) of patients. Figure 4A shows a summary of genetic alterations of VCAN in gastric cancer. In the 1365 sequenced STAD patients, genetic alteration was found in 115 patients and the mutation rate was 9% (Figure 4B). Kaplan-Meier plot and log-rank test showed that cases with gene alterations had longer DFS times than those without alterations (P=3.055e-3) (Figure 4C). These results show that genetic alterations in VCAN are associated with DFS of gastric cancer patients.

VCAN is a potential target for gastric cancer therapy:

In other to identify genes that are potential targets for treatment of malignant disorders, the open targets platform provides a means to score and rank target-disease associations by bringing together multiple data types to assist in the identification and prioritization of targets for further investigation(28). Surfing this platform, we found VCAN associated with gastric cancer and other diseases and the therapeutic area also reveals the possibility of VCAN as a target for gastric cancer therapy (Figure 5A – 5B).

Next, we constructed a network for VCAN with the structure or function of neighboring genes using GeneMANIA. The results showed that 20 genes – ANOS1, BCAN, CCL8, CD14, CD44, COL3A1, CXCL12, FBLN2, FBN1, HAPLN1, ITGAM, MMP9, SELL, SELP, SPARC, TLR1, TLR2, TLR6, TP53, TNFAIP6 – were closely associated with VCAN (Figure 6A).

Finally, we examined the interaction of VCAN protein with other human proteins using STRING database. The results revealed that VCAN interacts closely with 10 proteins – BCAN, BGN, CDH2, CHST11, CSPG4, DCN, FBN1, FN1, GPC1, and TLR2. Together, these results show that VCAN is a potential target for gastric cancer therapy; and also shows the neighbor gene interaction and protein interaction networks of VCAN.

Discussion

Gastric cancer has witnessed some advancements in terms of treatment, especially with the introduction of trastuzumab (a HER2 antibody) for the treatment of HER2 positive patients(32). However, only less than 20% of patients can benefit from this regimen(33) and as such gastric cancer is still one of the major causes of cancer-related deaths worldwide. Most deaths are due to lack of understanding of the genetic and molecular alterations and interactions that fuel tumorigenesis and progression; and non-availability of markers for detection, prediction of outcome, and targets for therapy.

In the present study, we investigated the role of VCAN in gastric carcinogenesis and found that VCAN is upregulated in gastric cancer and its expression was associated with, and increased with cancer stage. Overexpression of VCAN may have a role in initiation and progression of gastric cancer since changes were observed between normal and pathological stages and between each stage. VCAN may play an oncogenic role in gastric cancer, promoting disease progression from early to advance stage in accordance with several reports linking upregulated gene expressions with carcinogenesis and disease progression(34–40). Immunohistochemical examination of VCAN expression also confirmed our findings, with a higher protein expression in cancer tissues than normal tissues.

Elevated expression of VCAN predicted worse outcomes for patients as shorter survival times were observed in the high expression group compared with the low expression group. Again, previous evidence had documented the associations between upregulated gene expressions and poor patients outcome(41–44) and VCAN had been reported as an unfavorable prognostic marker in oral squamous cell carcinoma and endometrial cancer(11,45). VCAN expression in gastric cancer may be a potential diagnostic tool and a marker of poor outcome for patients.

Gastric cancer exhibits strong heterogeneity and varied sensitivity to chemotherapy drugs, with a strong need for the development of individualized therapies. Regimens available for the treatment of gastric cancer include (but not limited to) 5-fluorouracil (5-FU), platinum, taxane, irinotecan and anthracycline, with 5-FU being the most common(46,47). Selection of efficient chemotherapy drugs, design of optimal chemotherapy regimens and prediction of treatment response for gastric cancer requires comprehensive analysis and identification of chemotherapy-associated genes (47). We examined the effect of VCAN on various subgroups of patients especially with regard to treatment response. Interestingly, high VCAN expression correlated with worse outcomes for patients treated with surgery only or other adjuvants (other than 5-FU based adjuvant) only. However, patients expressing high VCAN who were treated with 5-FU based adjuvants had longer survival times than the low expression group. This shows that high expression of VCAN may be associated with chemosensitivity to 5-FU based adjuvants but chemoresistance to other adjuvants. VCAN expression may be applicable in determining appropriate treatment options for gastric cancer patients.

Gene alterations such as mutations, amplifications and deep deletions have been reported to influence cancer patients outcomes(31,48). We found 9% alteration of VCAN in gastric cancer and this was associated with better outcome in terms of DFS for patients. Hence, genetic alterations of VCAN may be a marker of favorable outcome for patients. Using the open targets platform also, we found VCAN associated with gastric cancer and the therapeutic area identified VCAN as a potential therapeutic target for gastric cancer. Neighbor genes interaction network revealed associations of VCAN with genes that function in tissue development (such as ANOS1, BCAN, FBLN2, and MMP9) and those that regulate metastasis by influencing cell-cell interactions, cell adhesion and migration (such as CD44, COL3A1, FBN1, HAPLN1, SELL, and SPARC), further showing the involvement of VCAN in tumorigenesis and disease progression. Interestingly also, VCAN closely interacted with a good number of immunity-related genes (such as CCL8, CD14, CXCL12, ITGAM, SELP, TLR1, TLR2, TLR6, and TNFAIP6). This is particularly

important because cancer is known to weaken the immune system, and there is evidence that regulatory T cells may be induced by tumors and downregulate the **immune system** response to **tumor** antigens, thereby incapacitating the immune system and facilitating disease progression(49). On the other hand, this association may be due to the tumorigenic nature of VCAN attracting enormous immune attention. We therefore hypothesize a possible VCAN-mediated immunogenicity and/or VCAN-mediated immune compromise during cancer progression. VCAN interactions at the protein level further validated this. VCAN interacted closely with proteins that function in tissue growth, development and regeneration (such as BGN, BCAN, and GPC1) and those involved in growth, adhesion and migration of tumor cells (such as CSPG4, FN1, FBN1, and CDH2) as well as TLR2 involved in innate and adaptive immunity.

This study had one major limitation - all the data analyzed were retrieved from online databases, and as such, further studies with larger sample sizes are required to validate our findings. Nevertheless, we demonstrated that VCAN is upregulated in gastric cancer and associated with clinical cancer stages. High VCAN expression predicted unfavorable outcome for patients, but better outcome for patients treated with 5-FU based adjuvant; and genetic alteration of VCAN is a positive marker for patients. Finally, VCAN is associated with genes and proteins that drive tumorigenesis and cancer progression, and has potential clinical application in the management and treatment of gastric cancer patients.

Conclusion

The results of this study show that VCAN is an oncogene in gastric cancer whose expression can be applied as a biomarker for prognostic prediction, selection of appropriate chemotherapy drugs/monitoring of treatment response, and is a potential therapeutic target for gastric cancer.

Abbreviations

5-FU: 5-fluorouracil; DFS: Disease-free survival; GEPIA: Gene expression profiling interactive analysis; HER2: Human epidermal growth factor receptor 2; OS: Overall survival, PPS: Post-progression survival; STAD: Stomach adenocarcinoma; TCGA: The Cancer Genome Atlas; VCAN: Versican.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Second Hospital of Shandong University.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available in the respective repositories [persistent web links to datasets and reference numbers are stated in the materials and methods section].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HBB, YW and CW conceived and designed the project. HBB prepared the figures and wrote the manuscript. HBB, YW, MAT, WL and LD analyzed/interpreted the data and revised the manuscript. All authors read and approved the final manuscript.

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Figures

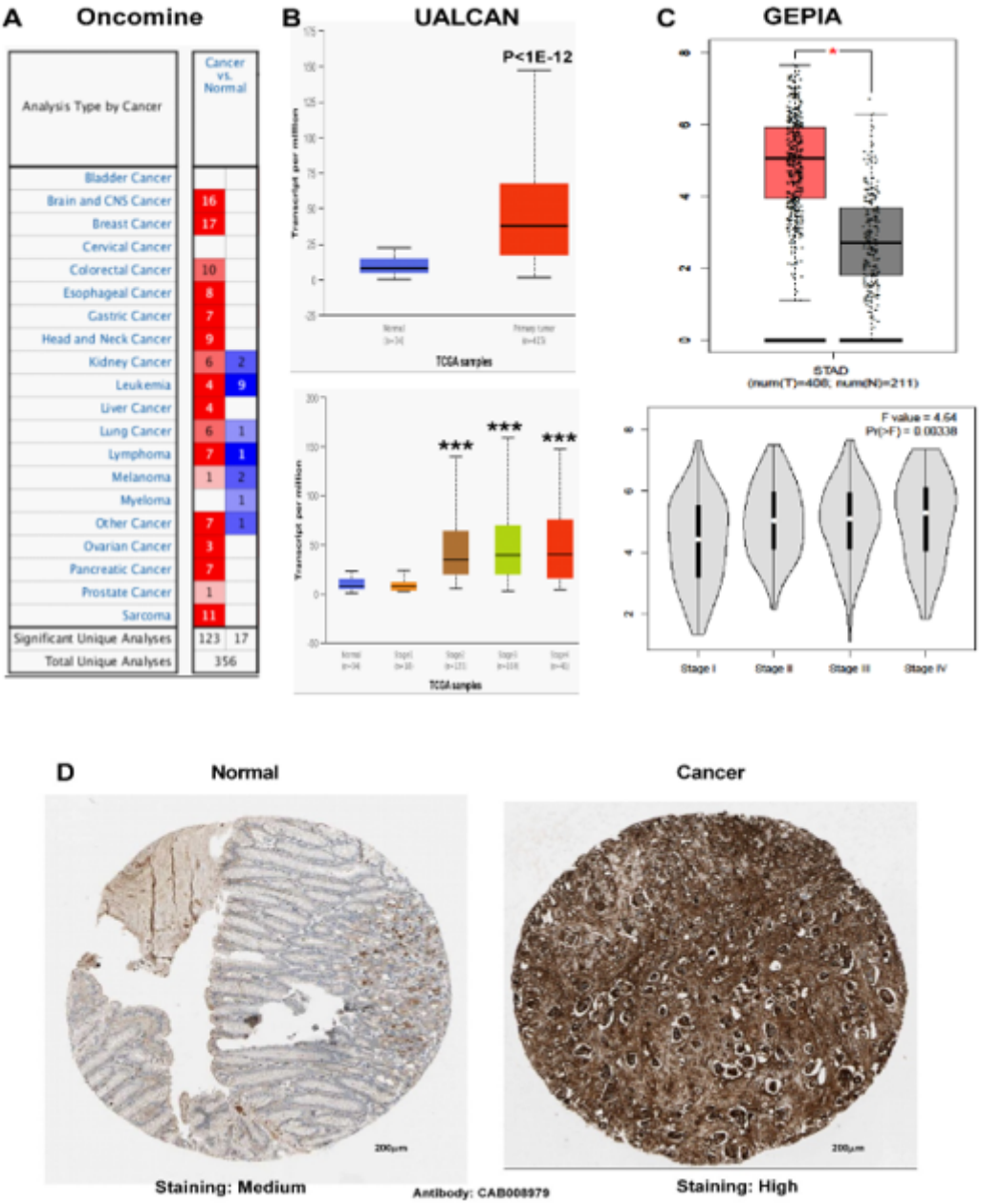

Figure 1

VCAN is upregulated in gastric cancer and associated with tumor stage; (A)The mRNA expression of VCAN in 20 different cancer types (cancer vs. normal tissue) was analyzed with ONCOMINE database. The number in each cell of the graph represents the number of datasets with statistically significant mRNA over-expression (red) or down-expression (blue) of the target gene. The P value threshold is 0.01. (B, C) VCAN is upregulated and associated with cancer stage in UALCAN and GEPIA databases. Its expression increases with cancer stage and the highest mRNA expression was found in stage 4. (D) Representative immunohistochemistry images of VCAN in human cancer tissues and normal tissues (Human Protein Atlas). VCAN protein was more highly expressed in cancer tissues than normal tissues. *P < 0.05, ***P < 0.001

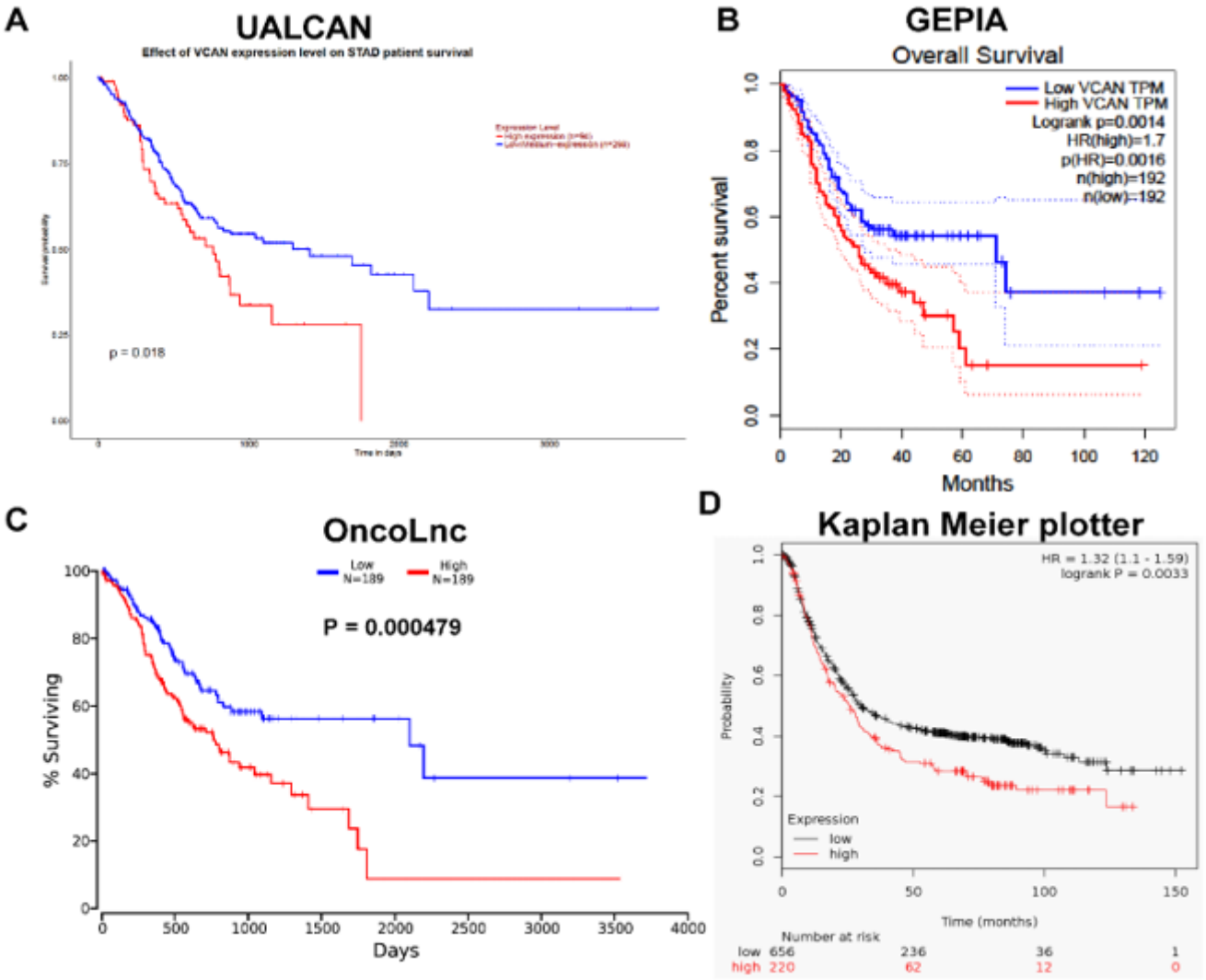

Figure 2

Prognostic value of VCAN in gastric cancer from four independent datasets. (A-D) High VCAN expression is associated with shorter survival times for all gastric cancer patients.

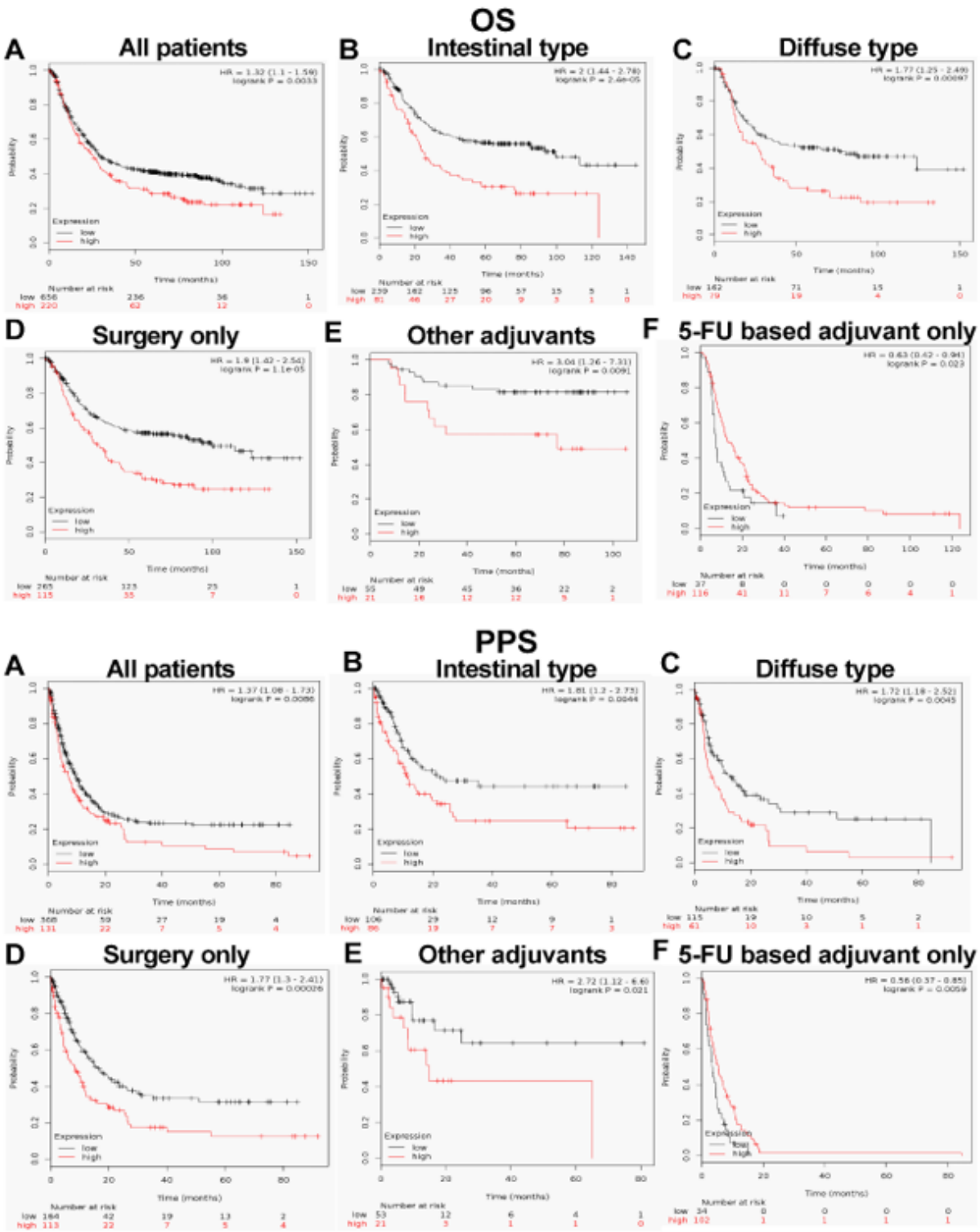

Figure 3

Subgroup analysis of prognostic value of VCAN in gastric cancer (Kaplan Meier Plotter). High VCAN expression predicted poor OS and PPS in (A) all patients, (B, C) intestinal type and diffuse type patients, (D, E) patients treated with surgery only and patients treated with other adjuvants only; but better OS and PPS in patients treated with 5-FU based adjuvant only (F).

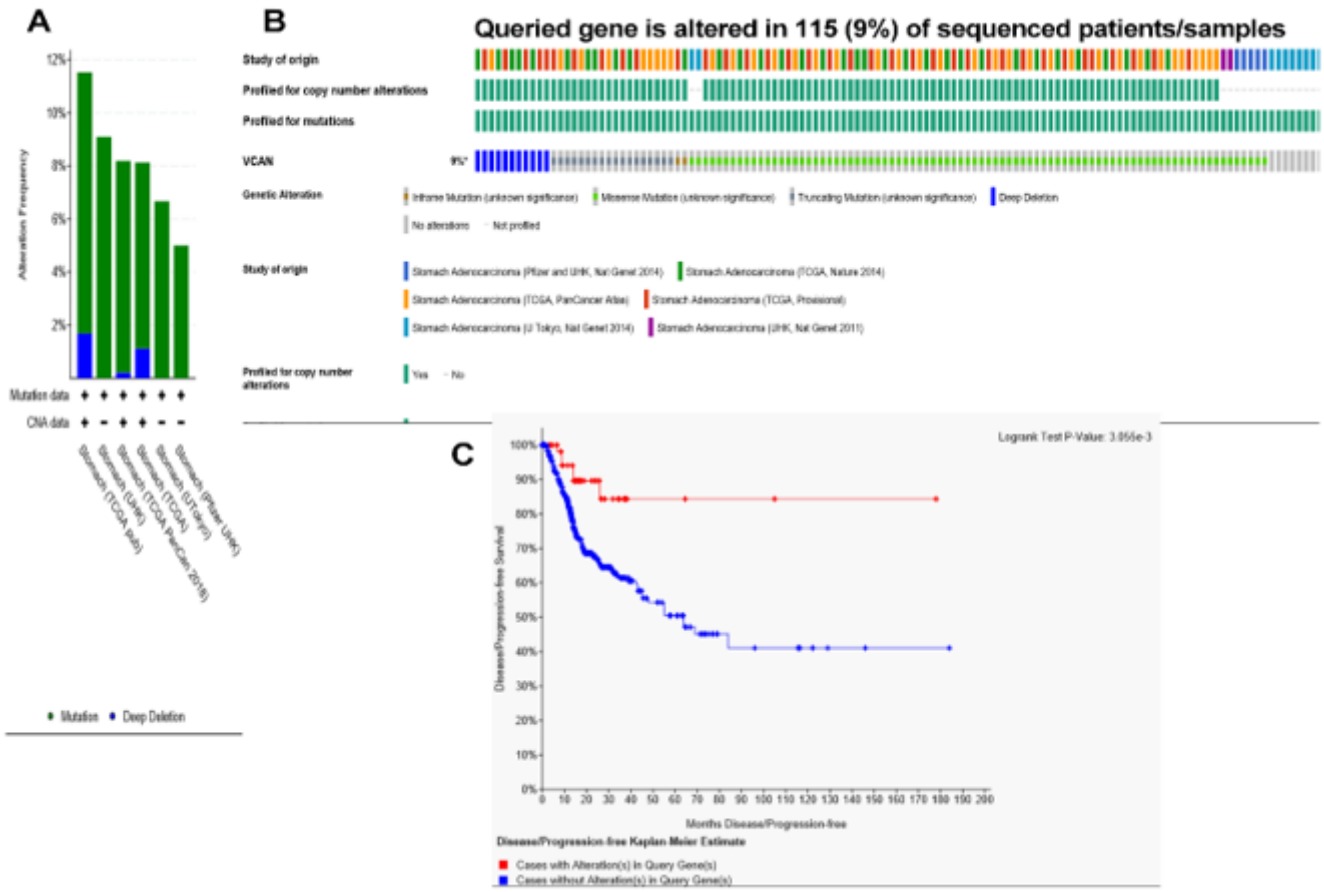

Figure 4

Genetic alterations in VCAN and its association with disease-free survival (DFS) of patients (cBioPortal). (A) summary of alterations in VCAN. (B) OncoPrint visual summary of alteration on a query of VCAN. (C) Kaplan–Meier plots comparing DFS in cases with/without VCAN gene alterations.

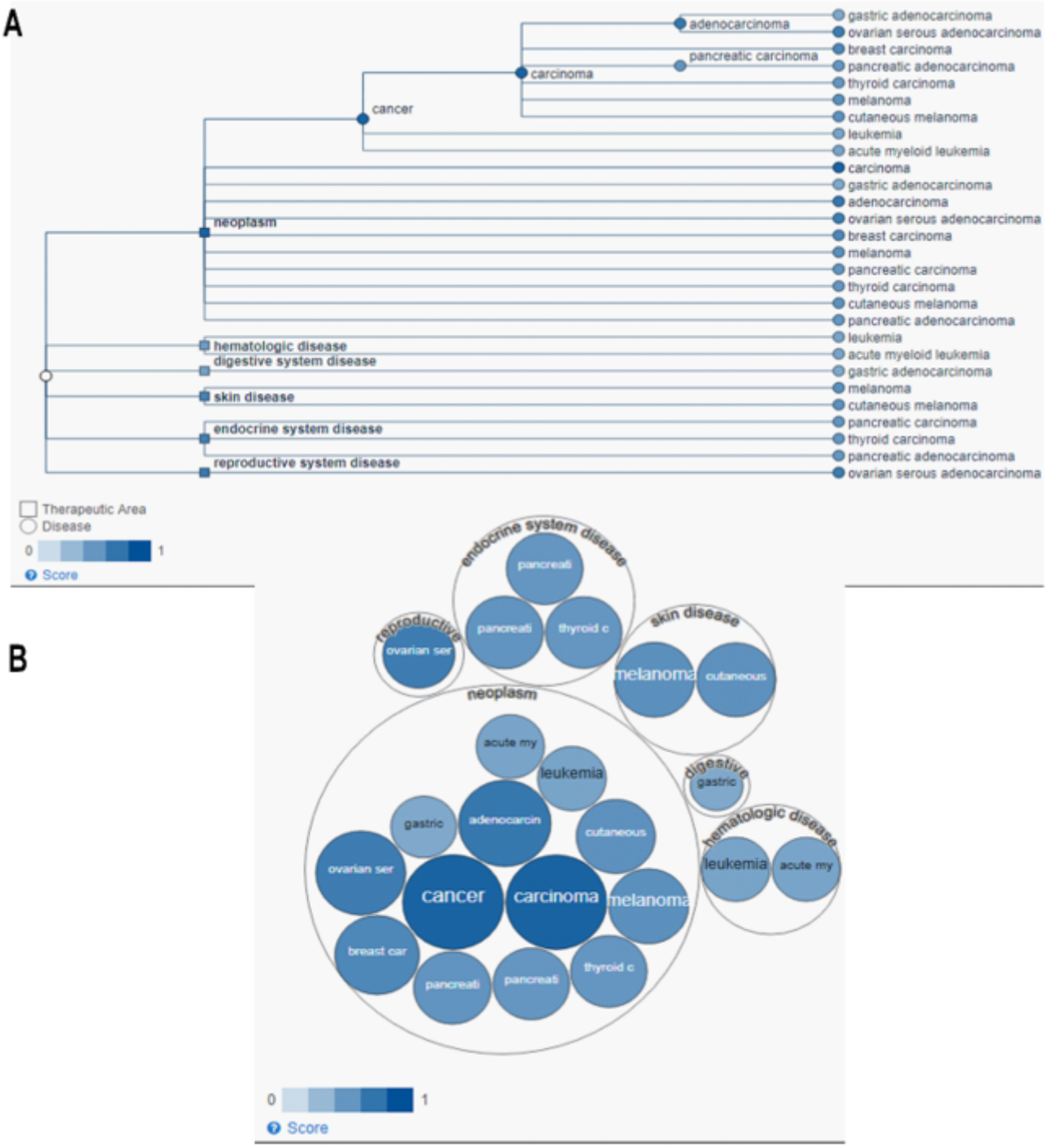

Figure 5

Diseases associated with VCAN based on pathways and systems biology (Open Targets Platform). (A, B) VCAN is associated with gastric cancer and other diseases based on pathways and systems biology.

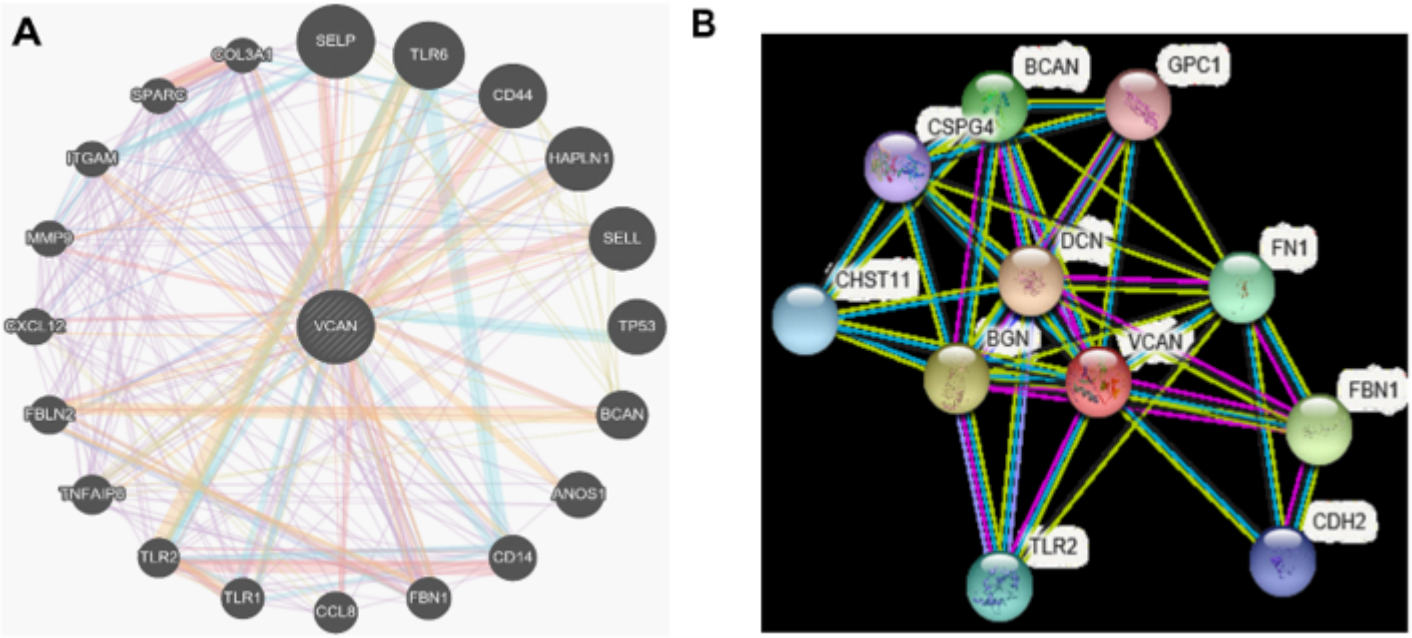

Figure 6

Neighbor gene interaction and protein-protein interaction networks of VCAN (GeneMANIA and STRING). (A) VCAN closely interacts with genes that function in tissue development (such as ANOS1, BCAN, FBLN2, and MMP9), those that regulate metastasis by influencing cell-cell interactions, cell adhesion and migration (such as CD44, COL3A1, FBN1, HAPLN1, SELL, and SPARC) and immunity-related genes (such as CCL8, CD14, CXCL12, ITGAM, SELP, TLR1, TLR2, TLR6, and TNFAIP6). (B) VCAN interacted closely with proteins that function in tissue growth, development and regeneration (such as BGN, BCAN, and GPC1) and those involved in growth, adhesion and migration of tumor cells (such as CSPG4, FN1, FBN1, and CDH2) as well as TLR2 involved in innate and adaptive immunity.